Kinetic Model of Biosurfactant-Enhanced Hexadecane Biodegradation by *Pseudomonas aeruginosa*

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Abstract: Many sites of environmental concern contain groundwater contaminated with nonaqueous phase liquids (NAPL). In such sites interfacial processes may affect both the equilibrium and kinetic behavior of the system. In particular, insoluble hydrocarbon partitioning and microbial biodegradation of insoluble hydrocarbon are influenced by the physicochemical and interfacial characteristics of the system. A mechanistic model describing the influence of biological surfactants on microbial biodegradation of liquid-phase insoluble hydrocarbon and subsequent reduction of nonaqueous-phase liquid hydrocarbon is presented. The model consists of six coupled differential equations which use lumped kinetic parameters to describe surfactant micelle formation and diffusion to the microbial cell, nonlinear kinetic expressions for microbial growth and degradation of insoluble hydrocarbon, kinetic spatial descriptions of the change in NAPL-phase droplet size and the organic phase volume fraction with time, as well as equilibrium partitioning expressions for hydrophobic organic contaminant partioning into the surfactant micelle. The model is validated by comparison to data obtained for hexadecane degradation in a well-mixed batch system by the biosurfactant producing microorganism *Pseudomonas aeruginosa* strain PG201 as well as for nonproducing mutants' growth and hexadecane biodegradation in the presence of exogenously added biosurfactant. Experimentally determined biological growth parameters, as well as physical parameters such as hydrocarbon droplet size, were applied in the kinetic model. Parameter sensitivity analysis was performed on the physical and biological parameters in the model. The parameter sensitivity analysis indicates that for the biological system examined the rate of hydrocarbon solubilization and micellar transport to the cell controls the rate at which cellular uptake and biodegradation of insoluble hydrocarbon occurs. Practical aspects relating to use of the model for support of surfactant-based bioremediation efforts are discussed. © 1999 John Wiley & Sons, Inc. *Biotechnol Bioeng* **63**: 401–409, 1999.

Keywords: NAPL reduction; biosurfactant; hexadecane; biodegradation; kinetic model; uptake mechanism

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INTRODUCTION

Many groundwater and aquifer contamination sites are characterized by the presence of multiple fluid phases. In such sites physicochemical phenomena and interfacial processes govern the phase partitioning of both the microorganisms and the organic compound. Remediation of hazardous waste sites often involves cleanup of hydrophobic organic contaminants (HOCs). For such compounds interfacial processes have a significant effect on their solubility and availability for treatment. These hydrophobic chemicals may be adsorbed, be dissolved in nonaqueous-phase liquids, or be present in a physically inaccessible state. Thus, many compounds that would normally be easily removed by traditional pump-and-treat methods or efficiently degraded by microorganisms are not removed because they are not readily available. The issues of contaminant solubility and availability have profound implications for assessment of site remediation strategies. The following areas have been identified as particularly relevant to hazardous waste site remediation: (1) delineation of the physicochemical mechanisms that affect the availability and transfer of hydrophobic organic compounds in heterogeneous, multimedia systems, (2) the rates of transformation of organic compounds in heterogeneous, multimedia systems, and (3) the limitations on microbial degradation of hydrophobic organic compounds that are sorbed to solids or that exist in organic liquid phases.

Mechanistic models have been recently developed that describe the influence of nonionic surfactants on the rate of solid-phase HOC dissolution under laminar flow conditions which may be useful in prediction of surfactant-enhanced transport and HOC dissolution in solid/aqueous-phase environments (Grimberg et al., 1995, 1996). We have developed a mechanistic model that describes the influence of biological surfactants on microbial biodegradation of liquid phase insoluble hydrocarbon and subsequent dissolution of nonaqueous-phase liquid (NAPL) HOC. The objectives of this study were (1) to derive a mechanistic kinetic mathematical model which predicts the rate of biosurfactant-mediated microbial biodegradation of insoluble hydrocarbon; (2) to validate the kinetic model using a biological

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experimental system consisting of biosurfactant-producing and nonproducing microorganisms; and (3) to perform parameter sensitivity analysis on the model to determine the most important physical and biological parameters in the model and relate them to mechanistic and environmentally relevant situations representing potential applications for the model.

BACKGROUND

Mechanisms of Microbial Uptake of Insoluble Hydrocarbon

Published reports support an integral role for biosurfactants in mediating the uptake and oxidation of insoluble hydrocarbon substrates by microorganisms. Biosurfactants have also been shown to enhance insoluble hydrocarbon partitioning into the bulk aqueous phase in multiphase systems containing soil, organic, and aqueous phases (Thangamani and Shreve, 1994). Several classes of biosurfactants are produced by microorganisms, usually in response to growth on insoluble substrates including certain hydrophobic organic contaminants (HOCs) such as naphthalene and phenanthrene (Cerniglia, 1984; Monticello et al., 1985), high molecular weight petroleum residuals (Finnerty et al., 1985), and branched and straight chain alkanes (Reddy et al., 1983; Singer and Finnerty, 1984; Vogt Singer and Finnerty, 1990). Microbial biosurfactants combine hydrophilic and lipophilic functional groups to form amphiphilic structures. The lipophilic portion is usually the hydrocarbon tail of one or more fatty acids often containing structural variations such as cyclic structures or hydroxyl functions. The polar portion of the biosurfactant may be a carbohydrate, amino acid, peptide, carboxylate, hydroxyl, phosphate group, or some combination of these functional groups. Most biosurfactants are either neutral or negatively charged. In the case of rhamnolipid, sophorolipid, and trehalose esters, the presence of biosurfactant has been shown to enhance the microbial degradation of hydrocarbons (Koch et al., 1991; Suzuki et al., 1969; Ito and Inoue, 1982; Breuil and Kushner, 1980; Goswami and Singh, 1991; Kosaric, 1993).

Three microbial mechanisms for the transport of insoluble hydrocarbon to the microbial cell have been widely described and are generally considered important for microbial growth. These include (1) transport and uptake of hydrocarbon molecules dissolved in the aqueous phase, (2) uptake via direct cellular contact with hydrocarbon droplets much larger than the cell, and (3) interaction of cells with pseudosolubilized or micellar-phase hydrocarbon. In the case of HOCs possessing an extremely low aqueous solubility, uptake of dissolved HOC (mechanism one) is believed to play a minor role in cellular growth. While it is apparent that biosurfactants play an important role in the uptake of certain insoluble hydrocarbons, it is currently unclear whether the observed kinetics of microbial biodegradation of insoluble hydrocarbon is governed predominantly by mechanism (2) or (3) or by a combination of both. These different processes may have a significant influence on degradation rates of organic compounds present in each phase and, hence, may be kinetically distinguishable. It has been proposed that charged and neutral biosurfactants may be involved in different cellular mechanisms of insoluble hydrocarbon uptake (Syldatk et al., 1984). Nonionic biosurfactants may render the charged cell surface hydrophobic, thereby facilitating the attachment and contact-dependent passive uptake of alkanes into the cell such as in the large droplet mechanism (Rapp et al., 1979; Ramsay et al., 1988). The large droplet mechanism of hydrocarbon uptake implies that the observed degradation rate is dependent on the area of the hydrocarbon-saturated surface or droplet/water interface. This area, however, is difficult to measure. Ionic biosurfactants such as rhamnolipids and sophorolipids may promote alkane uptake through solubilization of hydrocarbon in micelles allowing for bulk phase transport of the micellar hydrocarbon to the cell as described below (Fig. 1). For the mechanism involving micellar-phase transport, the observed hydrocarbon degradation rate should be a function of micellar-phase hydrocarbon concentration and therefore also of biosurfactant concentration. Evidence for both of these mechanisms exists (Ratledge, 1988; Kappeli and Finnerty, 1979; Bury and Miller, 1993).

Because of the difficulties in directly measuring interfacial areas and in quantifying biosurfactants/emulsifiers of often unknown structure, many previous studies have based their conclusions on biological models. The studies described here utilize a biological system consisting of rhamnolipid-producing *Pseudomonas aeruginosa* strain PG201 to validate a mechanistic kinetic model describing the influence of biosurfactant on the dissolution of nonaqueous-phase liquid (NAPL) hydrocarbon droplets into biosurfactant micelles and the rate of biosurfactant-enhanced biodegradation in well-mixed batch microbial systems (mechanism 3). The rhamnolipid produced by PG201 has been previously characterized as possessing a critical micelle concentration (CMC) of 18 mg/L resulting in a surface tension of 32 mN/m at the CMC (Thangamani and Shreve, 1994). *P. aeruginosa* strain PG201 and nonproducing mutants of PG201 were used to investigate the influence of cellular biosurfactant production as well as the influence of exogenously added biosurfactant on the microbial uptake of insoluble hydrocarbon and the ability of the mechanistic kinetic model presented to predict cellular growth and HOC biodegradation. This same cell system has been used previously to demonstrate the dependence of alkane degradation on rhamnolipid production (Koch et al., 1991; Ochsner, 1993). Nonproducing mutant 65E12 of *P. aeruginosa* is unable to grow in minimal media containing hexadecane as a carbon source in the absence of exogenously added surfactant. Mutant PG201::*rh1R* contains a Tn5 insertion which abolishes rhamnolipid synthesis, resulting in slow cell growth in the absence of exogenously added surfactants. Both mutants are deficient in the positive regulatory gene contolling the activation of rhamnolipid synthesis.

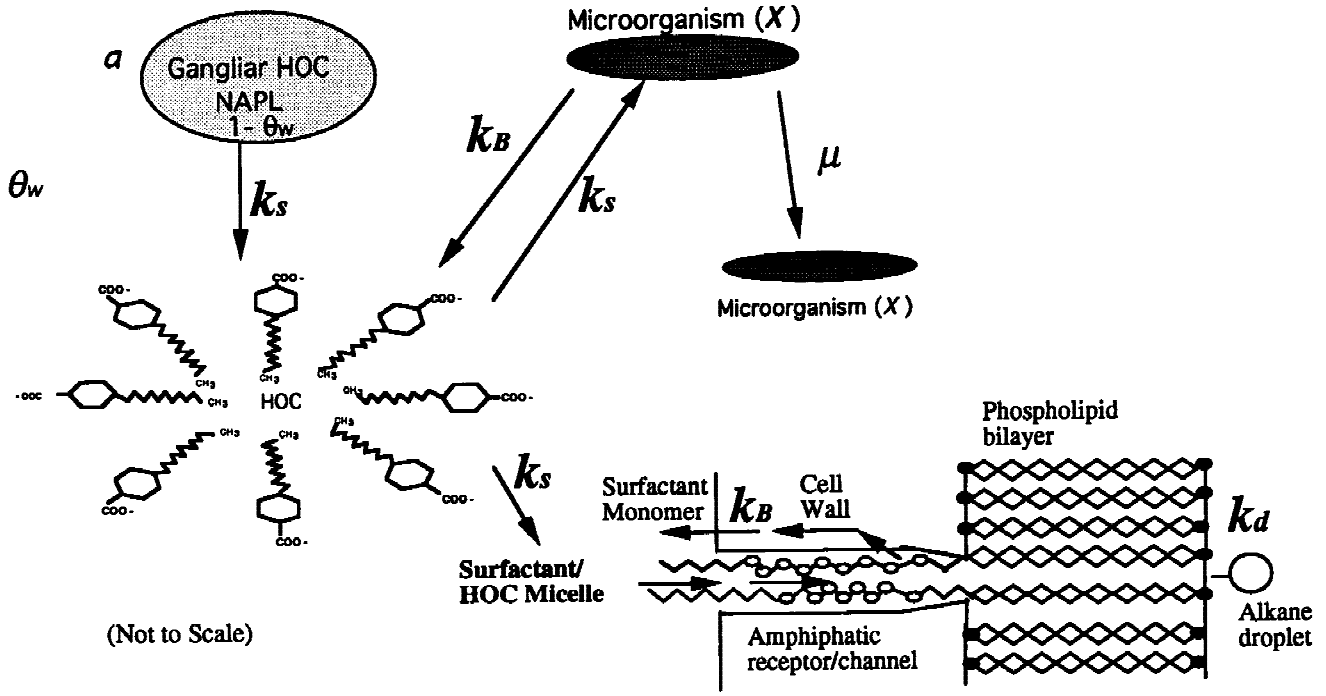

Figure 1. Schematic of surfactant-mediated microbial uptake of NAPL.

65E12 is a double mutant which is also deficient in lipopolysaccharide synthesis. However, growth of both mutants on hexadecane may be restored to varying degrees when small amounts of purified rhamnolipids or synthetic surfactants are added to the culture (Koch et al., 1991; Shreve et al., 1995).

Kinetic Modeling of NAPL Phase Reduction

The following mechanistic mathematical model was developed to describe the rate of NAPL source reduction in aqueous/organic systems due to biosurfactant-mediated microbial uptake of HOC. This model may also be applied to predict reduction of gangliar-free-phase NAPL material in the subsurface saturated zone. The model predicts microbial degradation of HOC, biosurfactant production, and microbial growth for biosurfactant producing microorganisms growing on a pure HOC in an organic/aqueous system as well as for exogenously added biosurfactant-facilitated microbial uptake of HOCs.

The foundation of the mathematical model is based on a mass balance of the NAPL-phase concentration in the aqueous/hydrocarbon system. Differentiation of the mass balance of the total NAPL-phase concentration with respect to time yields the change in total NAPL-phase concentration in the system with respect to time as

$$\frac{dC_{\text{tot}}}{dt} = \Theta_{\text{w}} \frac{dC_{\text{aq}}}{dt} + (C_{\text{aq}} - C_{\text{org}}) \frac{d\Theta_{\text{w}}}{dt} + (1 - \Theta_{\text{w}}) \frac{dC_{\text{org}}}{dt}, \quad (1)$$

where C_{tot} is the total concentration of NAPL HOCs in the system (mg/mL), C_{aq} is the concentration of the solubilized HOC (mg/mL), C_{org} is the total concentration of the organic constituent (HOC) in the multiphase system (mg/mL), and Θ_{w} is the fractional water content. The fractional water content is given as

$$\Theta_{\text{w}} = \frac{V_{H_2O}}{V_{H_2O} + V_{\text{org}}}, \quad (2)$$

where V_{H_2O} is the volume of water in the system and V_{org} is the volume of HOC phase in the system.

The change in concentration of solubilized HOC in the system is dependent on the rate at which the gangliar HOC is solubilized and degraded by the biomass in the system. The change in concentration of the solubilized HOC with respect to time is represented as

$$\frac{dC_{\text{aq}}}{dt} = a \frac{k_{\text{s}}}{\Theta_{\text{w}}} \left[\frac{B}{K_{\text{B}} + B} \right] - \left[\frac{k_{\text{d}} C_{\text{aq}}}{C_{\text{aq}} + K_{\text{dg}}} \right] \frac{X}{\Theta_{\text{w}}}, \quad (3)$$

where k_s is the specific rate of solubilization of the HOC from the NAPL phase in mg/(cm^2 h), B is the biosurfactant concentration (mg/mL), X is the biomass concentration (mg/mL), K_B is the half-saturation parameter for biosurfacant solubilization of HOC (mg/mL), a (cm^{-1}) is the ratio of the surface area of the organic-phase HOC droplet to the volume of the organic-phase HOC droplet, which when approximated as a spherical droplet is given by

$$a = 3/r, \quad (4)$$

where r is the radius of the average NAPL-phase ganglia in cm, k_d is the specific rate of degradation of solubilized HOC from the aqueous phase in h^{-1}, and K_{dg} is the half-saturation constant for the target HOC.

The first term in Eq. (3) represents the rate at which the gangliar HOC is solubilized by the biosurfactant in the system. The rate at which the gangliar HOC is solubilized is dependent on the surface area to volume ratio of the gangliar HOC. As the gangliar HOC droplet radius decreases, the surface to volume ratio of the gangliar HOC increases and the rate at which the gangliar HOC is solubilized increases. The rate of gangliar HOC solubilization is dependent on the fractional water content of the system, which increases as degradation of the HOC from the solubilized phase occurs. The gangliar HOC solubilization exhibits saturable kinetics with respect to the concentration of the biosurfactant in the system. This is represented by the $[B/(K_B + B)]$ term in Eq. (3). The fractional water content of the system increases due to the degradation of hexadecane by the microorganisms, and subsequently, the rate of solubilization of the gangliar HOC increases.

The second term in Eq. (3) represents the rate at which the solubilized HOC is degraded by the microorganisms. As the concentration of the biomass increases in the system due to growth, the magnitude of degradation of the solubilized HOC increases. The degradation of solubilized HOC also exhibits nonlinear kinetics which is represented by the $[C_{aq}/(C_{aq} + K_{dg})]$ term in Eq. (3).

As the gangliar NAPL droplets are solubilized, the gangliar droplet radius will decrease. Inspection of Eq. (4) shows that as the gangliar NAPL radius decreases, the surface area to volume ratio of the gangliar NAPL droplet increases. The change in the NAPL surface area to volume ratio with respect to time may be shown to be

$$\frac{da}{dt} = \frac{a^4 V_{H_2O}}{81} \frac{1}{\rho} \frac{dC_{aq}}{dt}, \tag{5}$$

where ρ is the density of the NAPL phase. The change in the NAPL surface area to volume ratio with respect to time is determined by differentiation of Eq. (4) with respect to time and subsequent substitution of the change in gangliar NAPL radius with respect to time. The density of the NAPL phase and the volume of water in the system are constant. As degradation of the solubilized HOC takes place, the change in solubilized HOC with respect to time and the surface area to volume ratio of the gangliar NAPL droplet increases. Therefore, the change in surface area to volume ratio of the gangliar NAPL droplet with respect to time increases.

Differentiation of Eq. (2) with respect to time results in an equation which contains a dV_{org}/dt term. The volume of the gangliar NAPL phase can be written as a function of the surface to volume ratio of the gangliar NAPL phase and substituted for V_{org}. The change in fractional water content in the system with respect to time is

$$\frac{d\Theta_w}{dt} = \frac{108\pi V_{H_2O}}{(V_{H_2O} + V_{org})^2 a^4} \frac{da}{dt}. \tag{6}$$

The change in the free biosurfactant concentration in the aqueous phase with time is dependent on the rate of biosurfactant production by the microorganism in the system and the rate at which biosurfactant is removed to form micelles which solubilize HOC. The change in free biosurfactant monomer concentration in the aqueous phase with time is given by

$$\frac{d[B]_{aq}}{dt} = \frac{k_B}{\Theta_w} X - \left(\frac{1}{MSR}\right) \frac{dC_{aq}}{dt}, \tag{7}$$

where k_B is the specific rate of production of biosurfactant by the biomass (h^{-1}), and MSR is the molar solubilization ratio. The MSR is calculated as the moles of a particular hydrocarbon solubilized per mole of biosurfactant present in concentrations above the CMC and is a direct measure of the ability of the biosurfactant to solubilize specific hydrocarbon species.

The change in biomass concentration due to growth from degradation of HOCs in the system with respect to time is represented by

$$\frac{dX}{dt} = \mu_{max} \left[\frac{C_{aq}}{C_{aq} + K_G}\right] X, \tag{8}$$

where μ_{max} is the maximum growth rate (h^{-1}), and K_G is the half-saturation parameter for biomass growth (mg/mL).

These equations were solved using Simusolv, a simultaneous differential equation solver. The ability of the model to predict HOC degradation and growth of the rhamnolipid producing strain *P. aeruginosa* PG201 and the nonproducing mutants 65E12 and PG201::*rh1R* in the presence of exogenously added biosurfactant was determined. The sensitivity of the model to the biological and physical input parameters was also examined.

EXPERIMENTAL METHODS

Microorganisms and Chemicals

P. aeruginosa PG201 and two mutants derived by transposon mutagenesis and designated *P. aeruginosa* 65E12 and PG201::*rhlR* were obtained from Jakob Reiser and Urs Ochsner at the Swiss Federal Institute of Biotechnology in Zurich. Rhamnolipid was also obtained from this source. Alkylbenzenesulfonate, tetrahydrofuran, and *n*-hexadecane were obtained from the Sigma Chemical Company. Tetrabutylammonium dihydrogen phosphate was obtained from Aldrich Chemical Company. Hexane was obtained from Fisher Scientific.

Microbiological Methods

The media used was a slightly modified version of the 4M medium described previously (Koch et al., 1991). KH_2PO_4 and K_2HPO_4 were used in the place of H_3PO_4 at concentrations of 4 and 5 g/L, respectively. In addition the following nutrients differed in concentration from the original 4M medium. The modified 4M media used contained 2 g/L $NaNO_3$, 0.5 g/L NaCl, 0.5 g/L KCl, 0.025 g/L $CaCl_2$, 0.25

mg/L $FeSO_4$, 0.45 mg/L, H_3BO_3, 0.75 mg/L $ZnSO_4 \cdot 7H_2O$, and 0.75 mg/L $MnSO_4 \cdot H_2O$. In all experiments with wild type PG201 cells, hexadecane was added to the minimal media at a starting concentration of approximately 0.9 mg/mL. Glycerol stock cultures (20% v/v) were made in various media including minimal media containing hexadecane and were stored at −20°C. Glycerol stocks were subcultured once every 2 months. Solid stocks in agar were also made. Liquid culture cell concentrations were determined by plate counts of serially diluted samples on Petri plates containing agar and tryptone nitrate media, and these were related to total protein using the Lowry method for protein determination (Lowry et al., 1951). The OD_{750} obtained was related to the number of cells present per milliliter of culture through previous standardizations of protein content determination per cell, using plate counts.

Batch Kinetic Experiments

Batch kinetic experiments were conducted in 50-mL serum bottles containing 0.9 mg/mL of hexadecane in bacteriological media with a total liquid volume of 20 mL and containing approximately 30 mL of headspace. For each experiment a number of 50-mL serum bottles were set up identically. Each serum bottle contained exactly the same amount of hexadecane, minimal media, and inoculum, which was added at the beginning of the experiment. The 50-mL serum bottles were sealed with Teflon-coated stoppers. The pH of the media was constant throughout the experiment at approximately 6.4. Cultures were shaken at 200 rpm and maintained at a temperature of 33°C. Due to the negligible solubility of hexadecane in the aqueous phase it was not possible to obtain a uniform sample from the two-phase system for analysis, hence, a sacrificial bottle technique was used in all the experiments. At every sampling time point one triplicate set of bottles was sacrificed for analysis. The average hexadecane and biosurfactant concentrations and the associated experimental standard deviations were calculated. In batch experiments with the biosurfactant producing wild type strain PG201, 2 mL of well-mixed culture was removed prior to hexane extraction and subjected to HPLC analysis for total rhamnolipid concentration as described below. The remaining culture contents were extracted into an equal volume of hexane (1:1 extraction ratio). After addition of the hexane phase the bottles were tightly sealed with Teflon-coated stoppers and aluminum crimp caps. They were inverted and manually shaken for 3–5 min and then placed on a rotary shaker at 100 rpm for about 2 h. The extraction efficiency of this procedure was determined to be 100% over the experimental concentration range prior to analysis of experimental samples. This was accomplished by extracting known quantities of hexadecane, in triplicate batch extractions, in a six-point extraction calibration procedure spanning the 0 to 0.9 mg/mL hexadecane concentration range.

Hexadecane Analysis

Hexadecane was analyzed on a Perkin Elmer Autosystem GC using a flame ionization detector. A Restek Rtx-1, 0.53 mm I.D. megabore column with a stationary phase thickness of 3 μm was used for the hexadecane analysis. Helium was used as the carrier gas at a flow rate of 8 mL/min. The over temperature program started at an initial temperature of 125°C, held at this temperature for 0.5 min, and then proceeded with a single temperature ramp from 125 to 167°C at a rate of 10°C/min. The flow rates of hydrogen and air were 40 and 400 mL/min, respectively. The injector temperature was held at 280°C, and the detector temperature was held at 290°C. The total hexadecane concentration in each experimental bottle was measured by extracting the serum bottle contents with 20 mL of pure HPLC grade hexane. Triplicate 1-mL samples of the hexane phase were then analyzed on the GC to determine the average hexadecane concentration. The GC precision on these samples was 1–2%.

Rhamnolipid Analysis

The quantity of rhamnolipid produced by the wild type PG201 cultures was analyzed on a Waters LC Module-1 HPLC. A C-18 Novapak column was used in this study. The mobile phase consisted of 60% water and 40% tetrahyrofuran containing paired ion chromatography (PIC A) reagent tetrabutylammonium dihydrogen phosphate at a concentration of 0.005 *M*. The mobile phase flow rate was 1 mL/min. A Waters 486 UV detector was used to detect the rhamnolipid at a wavelength of 254 nm. An aqueous sample from each experimental bottle was centrifuged, and the supernatant was filtered through a 0.22 μm Acrodisc filter to remove cell debris. A 50-μL amount of the filtered sample was then analyzed in the HPLC for rhamnolipid. HPLC was performed prior to analysis of experimental samples with triplicate samples of prepared rhamnolipid samples in culture media spanning the concentration range examined under experimental conditions; the rhamnolipid samples were filtered identically to experimental samples. Triplicate samples were analyzed at each concentration; physical losses for the filtration step were approximately 10%, and experimental sample concentrations were corrected for these losses.

RESULTS

Experimental and Kinetic Model Results for Hexadecane Degradation and Microbial Growth

Microbial uptake of hexadecane was evaluated by monitoring hexadecane disappearance in the experimental cultures. Since no abiotic losses of hexadecane occurred and hexadecane was the sole carbon and energy source of microbial growth, the hexadecane disappearance rate directly reflects the microbial uptake and subsequent catabolic degradation

of hexadecane (Koch et al, 1991). The micellar phase concentration of hexadecane was determined by multiplying the concentration of rhamnolipid by its molar solubilization ratio (MSR). The MSR of the rhamnolipid is 5.74 mg of hexadecane solubilized per milligram of rhamnolipid biosurfactant (Shreve et al., 1995).

Simusolve was used to examine the ability of the kinetic model to predict hexadecane degradation and cell growth for three sets of experimental data. One data set was from the PG201 biosurfactant-producing strain and two additional data sets were, respectively, from the mutant nonproducing strains 65E12 and PG201::*rh1R*. Whenever possible the input parameters for the model were measured from the experimental data. The measured and fitted experimental kinetic parameters are summarized in Table I. The ability of the mathematical model to predict the experimental data to within one experimental standard deviation for each of the time points was examined.

For most of the PG201 batch experiments the hexadecane uptake rate increased slightly over the degradation period while the concentration of rhamnolipid increased roughly in proportion to cell mass over the period. While cellular biomass increases roughly with Monod type nonlinear kinetics (Eq. (8)), it was not possible to fit the biomass data precisely when Eq. (8) was coupled and solved together with the other five nonlinear equations within the model. It is also clear from the biosurfactant and biomass data (Fig. 2) that the total biosurfactant concentration in the system increases in direct proportion to biomass. Since Eq. (7) represents a mass balance on aqueous-phase biosurfactant, the model and associated kinetic parameters predict an initial increase in aqueous-phase monomer as biosurfactant monomer is produced and then a decrease as biosurfactant monomer is removed from the system via micelle formation. The measurement of aqueous-phase free monomer is somewhat difficult, requiring ultrafiltration or other methods, whereas the sacrificial bottle technique used in the experiments described measures total biosurfactant in the system including micell-associated and free monomer. Therefore, only the first term in Eq. (7) representing the biosurfactant production was applied in examining the experimental data collected (Fig. 2). The first-order rate constant (k_B/θ_w) is not actually constant since θ_w changes with time from 0.95 to 1.0, hence the calculated rate constant varies by 5% over time.

The experiments for 65E12 and PG201::*rh1R* were conducted in the presence of 0.1 mg/mL of exogenously added biosurfactant. Since the mutant microorganisms 65E12 and PG201::*rh1R* are unable to produce biosurfactant, the concentration of biosurfactant, in the absence of degradation, will remain constant, hence, Eq. (7) was set equal to zero in the model and the biosurfactant concentration was held constant. The results of the simulation for 65E12 (Fig. 3) indicate a lower solubilization rate constant as well as a lower observed degradation rate over time than that of PG201::*rh1R* (Fig. 4).

Parameter Sensitivity Analysis

A parameter sensitivity analysis was performed on the mathematical model relative to its fit to the *P. aeruginosa* PG201 strain data. The ability of the model to predict biomass and hexadecane concentrations at the last experimental data point taken at 46.5 h was investigated for varying values of the model input parameters: a_o, k_B, k_s, μ_{max}, k_d, and K_{dg}. One kinetic parameter was varied as the remaining parameters were held constant at the values shown in Table I. The results of the parameter sensitivity analysis are summarized in Table I. The parameter sensitivity analysis indicated which kinetic parameters most affected the ability of

Table I. Parameter sensitivity analysis results.

Parameter	PG201 simulation value[a]	Value range[b]	X (mg/mL)[b]	C (mg/mL)[b]
Experimental Data			0.539	0.382
Model Predictions			0.749	0.377
a_o (cm^{-1})	42 (M)	25 (*−40*)	0.051 (*−93*)	0.628 (*+67*)
		44 (*+5*)	1.195 (*+60*)	0.322 (*−15*)
k_B (h^{-1})	0.361 (F)	4×10^{-5} (*−100*)	0.735 (*−2*)	0.381 (*+1*)
		40 (*+10,000*)	0.812 (*+8*)	0.372 (*−1*)
k_s (mg cm^{-2} h^{-1})	9.335×10^{-5} (F)	4×10^{-5} (*−57*)	0.025 (*−97*)	0.707 (*+88*)
		1×10^{-4} (*+7*)	1.148 (*+53*)	0.353 (*−6*)
μ_{max} (h^{-1})	2.1208 (M)	1.0 (*−53*)	0.032 (*−96*)	0.313 (*−17*)
		2.8 (*+32*)	3.404 (*+354*)	0.621 (*+65*)
k_d (hr^{-1})	7.334×10^{-3} (F)	7×10^{-4} (*−90*)	0.882 (*+18*)	0.313 (*−17*)
		0.07 (*+850*)	0.278 (*−63*)	0.720 (*+91*)
K_{dg} (mg/mL)	7.808×10^{-5} (F)	8×10^{-6} (*−90*)	0.729 (*−3*)	0.379 (*+1*)
		8×10^{-4} (*+924*)	0.741 (*−1*)	0.379 (*+1*)
K_G (mg/mL)	1.5 (M)			
K_B (mg/mL)	0.0012			

[a]M, measured; F, fitted.
[b]Percent change from initial value indicated in parentheses.

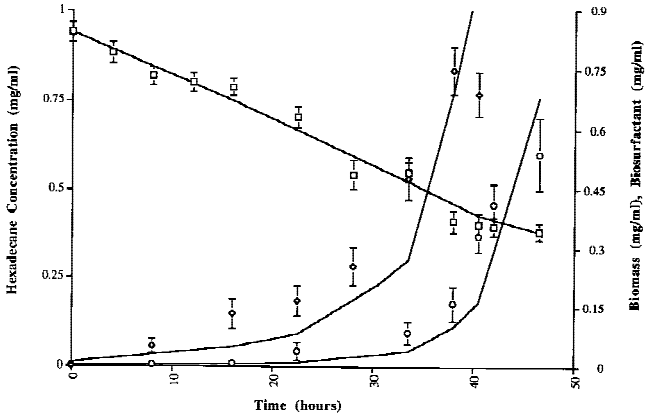

Figure 2. Data and kinetic model of rhamnolipid-mediated hexadecane degradation by *P. aeruginosa* PG201. (□) Hexadecane concentration (mg/mL); (○) biomass concentration (mg/mL); (◇) biosurfactant concentration (mg/mL).

the model to predict hexadecane degradation and microbial growth. Table I indicates how parameter changes affected the ability of the model to predict the PG201 biomass concentration (X) and hexadecane concentration (C_T) experimental values at an elapsed time of 46.5 h. Parameters were varied one at a time, generally by 1 to 2 orders of magnitude where not limited by convergence of the simultaneous solution of the differential equations described. For predicting the biomass concentration, the model is most sensitive to parameter changes of a_o, k_s, μ_{max}, and k_d and less sensitive to parameter changes of k_B and K_{dg}. Examining the model's ability to predict the hexadecane concentration, we determined that the model is most sensitive to changes of a_o, k_s, μ_{max}, and k_d and less sensitive to changes in k_B and k_{dg}. Decreases in a_o and k_s affect the ability of the model to fit the hexadecane concentration more than increases in these parameters, in which the fit is relatively unaffected. Increases in μ_{max} and k_d affect the ability of the model to fit

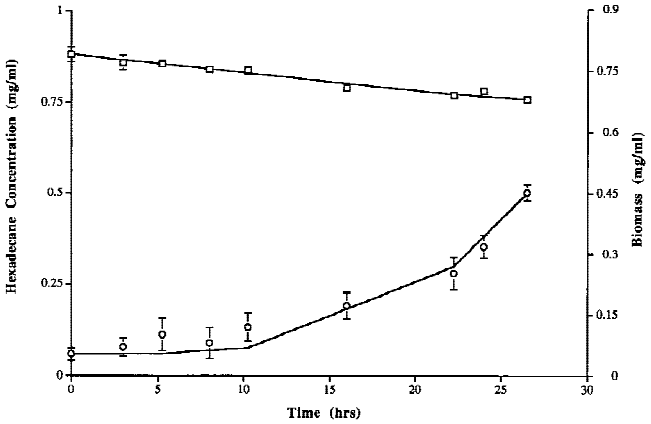

Figure 3. Data and kinetic model results for PG201::*rh1R*. (□) Hexadecane concentration (mg/mL); (○) biomass concentration (mg/mL). (Fitted with $a_o = 42\ cm^{-1}$, $K_G = 2.243 \times 10^{-4}$ mg/mL, $K_B = 0.0012$ mg/mL, $k_s = 5.845 \times 10^{-5}$ mg cm^{-2} h^{-1}, $\mu_{max} = 0.1444\ h^{-1}$, $k_d = 4.421 \times 10^{-8}$ h^{-1}, and $K_{dg} = 0.092$ mg/mL).

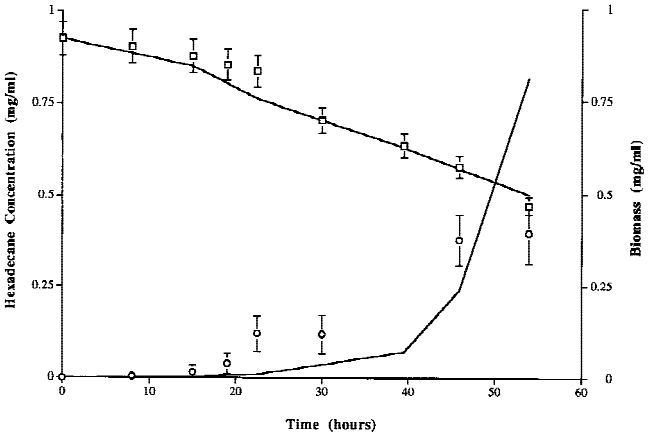

Figure 4. Data and kinetic model results for 65E12. (□) Hexadecane concentration (mg/mL); (○) biomass concentration (mg/mL). (Fitted with $a_o = 42\ cm^{-1}$, $K_G = 0.560$ mg/mL, $K_B = 0.0012$ mg/mL, $k_s = 3.820 \times 10^{-5}$ mg cm^{-2} h^{-1}, $\mu_{max} = 2.1224\ h^{-1}$, $k_d = 1.894 \times 10^{-5}\ h^{-1}$, and $K_{dg} = 5.294 \times 10^{-10}$ mg/mL.)

hexadecane concentration more than decreases in these parameters, in which the fit is relatively unaffected.

DISCUSSION

The ability of the proposed kinetic model to fit the behavior of the PG201 and mutant systems described indicates that for these *Pseudomonas* strains uptake of NAPL-phase hexadecane may be predicted using a kinetic model describing microbial uptake via micellar solubilization and subsequent biodegradation. These results are consistent with previous observations of the effect of rhamnolipid on hexadecane uptake by *P. aeruginosa* (Koch et al., 191; Goswami and Singh, 1991). The relatively good fit of the model to the data supports the idea that microbial uptake of hydrocarbon directly through the hydrocarbon/water interface is a secondary mechanism of hydrocarbon transport for the microbial system examined since this mechanism is not included in the kinetic model presented. Perhaps over the 24 to 48 h period in which most of our kinetic measurements were taken little change in the cell's ability to localize at hydrocarbon interfaces occurred. Inclusion of this mechanism in the kinetic model would not be mathematically difficult; however, a precisely known value for the total organic/aqueous interfacial area would be required. Alternate proposed and previously discussed mechanisms are also not described by this kinetic model (Zhang and Miller, 1994, 1995).

The observed sensitivity of the model to variation of the initial surface area to volume ratio (a_o) of the gangliar HOC droplet may indicate that the level of agitation in the system is important. A more agitated system will contain an increased amount of gangliar HOC droplets. Therefore, the surface area to volume ratio of the system will be larger. The increase in gangliar surface area may aid in biosurfactant micelle formation, which may subsequently aid in the

biological uptake of the HOC. The sensitivity analysis indicates that an increased surface area to volume ratio results in a lower HOC concentration prediction at 46.5 h.

The sensitivity of the model to variation of the specific rate of solubilization and transport of the micellar HOC to the microbial cell (k_s) may indicate that k_s is the limiting rate for biological degradation of HOC by the biosurfactant-enhanced mechanism. The sensitivity analysis indicated a significant dependence of the model on the solubilization and transport parameter (k_s) with the model predicting a very rapid decrease in the HOC concentration as the specific rate of solubilization was increased. The model parameter k_s is a lumped kinetic parameter that represents the surfactant solubilization of HOC and subsequent transport of micellar phase HOC to the cell. Previous models and studies have also discussed the potential for surfactant micelle formation on the surface of the NAPL droplet with subsequent diffusion of the HOC containing micelle through the hydrodynamic boundary layer for being the rate-limiting process in insoluble hydrocarbon dissolution (Grimberg et al., 1995, 1996). Subsequent diffusion of the HOC-containing micelle through the bulk aqueous phase and contact with the microorganisms are also required for HOC biodegradation. At the cellular level the apparent degradation rate constant, k_d, represents the rate-limiting step in the processes of hydrocarbon uptake and biodegradation which may be directly coupled depending on the cell's physiological state. The parameter sensitivity analysis performed on our model strongly indicates that the rate at which the solubilization and micellar transport to the cell occurs may dictate the rate at which cellular uptake and biodegradation of HOC occurs in the experimental system under examination.

Most NAPL-contaminated field sites contain hydrocarbon mixtures. A model such as the one presented here may be extended to describe the solubilization and biodegradation of such mixtures if the relevant model input parameters are known. Determination of such individual parameters for each hydrocarbon species as well as the specific biosurfactant and microbial system would be laborious. Additional confounding factors in extending the model presented to a multicomponent field model involving microbial consortia would include effects such as changes in the MSR of a biosurfactant for a specific HOC in the presence of additional HOCs. The addition of various hydrocarbon species to the experimental system may affect the organic character of the hydrophobic core of the micelle. Also, the biological solubilization, HOC degradation, growth, and biosurfactant production rate constants will reflect the characteristics of the mixed consortia of microorganisms present within the site. Under such conditions the kinetic parameters and equilibrium parameters used (MSR) should be lumped parameters which are determined for the specific population of organisms and insoluble HOC mixture under examination. Previous studies suggest that physiological conditions for growth of biosurfactant-producing microorganisms may influence their structure, and hence, effectiveness in given applications as well as introduce the probability that these parameters will not remain constant as microbial populations evolve within the site (Falatko and Novak, 1992; Finnerty, 1992). While the model also adequately predicts the growth and hydrocarbon biodegradation kinetics for nonproducing microorganisms in the presence of exogenously added biosurfactant, the selection of microorganisms capable of producing biosurfactants in situ may be superior to the addition of exogenous surfactant. This is because the solubilization and transport (k_s) parameter may be significantly different for biosurfactant which is produced by microorganisms in the proximity of the NAPL phase versus that for exogenously added surfactant which must diffuse and become distributed at the aqueous/organic interface. In addition, k_s may be significantly smaller in three-phase systems containing soil where the presence of the soil phase may influence biosurfactant partitioning as well as micellar diffusion to the cell.

In summary, the mechanistic kinetic model presented will be useful for understanding the processes which may be rate limiting for NAPL source reduction via surfactant solubilization and microbial degradation. This model may be expanded to include surfactant solubilization and HOC degradation by microbial populations in organic/aqueous/soil-containing multiphase systems. This type of kinetic model may be modified to fit into other existing multiphase contaminant transport models to further predict insoluble hydrocarbon persistence and mobility, allowing for improved decision making and support of surfactant-based bioremediation strategies.

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NOTATION

a	surface to volume ratio of HOC gangliar-phase spherical droplet (cm^{-1})
a_o	initial surface to volume ratio of HOC gangliar-phase spherical droplet (cm^{-1})
B	biosurfactant concentration (mg/mL)
C_{aq}	concentration of the solubilized HOC (mg/mL)
C_{org}	total concentration of organic constituent (HOC) in the multiphase system (mg/mL)
C_{tot}	total concentration of NAPL HOCs in the system (mg/mL)
k_B	specific rate of production of biosurfactant by the biomass (h^{-1})
K_B	half-saturation parameter for biosurfactant solubilization of HOC (mg/mL)
k_d	specific rate of degradation of solubilized HOC from the aqueous phase (h^{-1})
K_{dg}	maximum degradation rate constant for the target HOC (mg/mL)
K_G	half-saturation parameter for biomass growth (mg/mL)
k_s	lumped parameter describing the rate of solubilization of the HOC from the NAPL phase and transport of micellar phase HOC to the cell (mg cm^{-2} h^{-1})

MSR	molar solubilization ratio (dimensionless)
r	radius of spherical HOC gangliar-phase spherical droplet (cm)
t	time (h)
V_{H_2O}	volume of water in the system (mL)
V_{org}	volume of the HOC phase in the system (mL)
X	biomass concentration (mg/mL)

Greek Letters

μ_{max}	maximum growth rate of the biomass (h^{-1})
ρ	density of the HOC (mg/mL)
θ_w	fractional water content of the system (dimensionless)

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